Screening, Prep-HPLC Isolation and Chemical Characterization of Potential Bioactive Compounds Derived from *Selaginella Bryopteris* Using Advanced Analytical Tool

SHORT SYNOPSIS

Submitted in partial fulfillment of the requirement of the degree of

DOCTOR OF PHILOSOPHY

by

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MANAV RACHNA UNIVERSITY

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DECLARATION BY THE SCHOLAR

I hereby declare that the short synopsis entitled **"Screening, Prep-HPLC Isolation and Chemical Characterization of Potential Bioactive Compounds Derived from *Selaginella Bryopteris* Using Advanced Analytical Tools"** being submitted in partial fulfillment of the requirement for the degree of Doctor of Philosophy in the Department of Sciences under the School of Sciences, Manav Rachna University, Faridabad during the academic year 2025-2026.

It is the work that is expected to be carried out under the supervision of **Dr. Roopa Rani,** Assistant Professor, Department of Sciences, School of Sciences, and co-supervision of **Dr. Muralidharao Bagadi,** Director Analytical Department, Jubilant Biosys., Greater Noida and **Dr. Pradeep K. Varshney**, Professor, Director Academics and Quality Assurance, Swami Rama Himalayan University, Dehradun.

Further, it is declared that the short synopsis is within the plagiarism limit and copy of the same is attached as per the University norms.

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CERTIFICATE BY SUPERVISOR(S)

This is to certify that, the short synopsis entitled **"Screening, Prep-HPLC Isolation and Chemical Characterization of Potential Bioactive Compounds Derived from *Selaginella Bryopteris* Using Advanced Analytical Tools"** is an original piece of work done by research scholar **Ms. Sapna Sharma** under the supervision of **Dr. Roopa Rani,** Assistant Professor, Department of Sciences, School of Sciences and co-supervision of **Dr. Muralidharao Bagadi,** Director Analytical Department, Jubilant Biosys., Greater Noida and **Dr. Pradeep K. Varshney**, Professor, Director Academics and Quality Assurance, Swami Rama Himalayan University, Dehradun for the degree of Doctor of Philosophy in the Department of Sciences under the School of Sciences, Manav Rachna University, Faridabad during the academic year 2025-2026.

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ABSTRACT

Phytochemicals are bioactive substances that exist naturally in plants. The *Selaginella Bryopteris* is a member of the family *Selaginellaceae*, which is also referred to as "Sanjeevani." This research aims to explore the development of new analytical methods to improve isolation, purification and structure elucidation of potential bioactive compounds of *Selaginella Bryopteris* through comprehensive study. The study shall include preparative HPLC for the effective isolation of the plant's constituents, followed by detailed structural elucidation using NMR and Hyphenated-MS techniques. Furthermore, AI tools shall be used to predict the biological activities of the isolated compounds, focusing on potential antimicrobial, anti-inflammatory, antioxidant and oncological effects. This innovative approach seeks to bridge traditional knowledge and improves the understanding with modern technology, potentially uncovering novel Phytopharmaceuticals and enhancing our understanding of *Selaginella Bryopteris* therapeutic applications in modern era.

Keywords: Phytochemicals, *Selaginella Bryopteris*, isolation, Prep HPLC, NMR, MS.

LIST OF ABBREVIATIONS

SM: Secondary Metabolites

HPLC: High-Performance Liquid Chromatography

NMR: Nuclear Magnetic Resonance

MS: Mass Spectrometry

WHO: World Health Organization

TM: Traditional Medicine

AI: Artificial Intelligence

AOAC: Association of Official Analytical Chemists

TLC: Thin Layer Chromatography

GC: Gas Chromatography

UV-VIS: Ultraviolet-Visible

FT-IR: Fourier Transform Infrared

ROESY: Rotating-frame Overhauser Effect Spectroscopy

IC50: Half Maximal Inhibitory Concentration

PPM: Parts Per Million

SPE: Solid Phase Extraction

Ag-NPs: Silver Nanoparticles

S. Bryopteris: Selaginella Bryopteris

CSIR: Council of Scientific and Industrial Research

TFCS: Total flavonoid content

Mg: Milligram

Kg: Kilogram

1D: One-Dimensional

2D: Two-Dimensional

HRMS: High-Resolution Mass Spectrometry

LIST OF TABLES

Table no.	Description	Page no.
Table 1	Taxonomical classification of *Selaginella Bryopteris*	2
Table 2	List of methods used for extraction, isolation and identification of plant *Selaginella Bryopteris*	9

LIST OF FIGURES

Figure no.	Description	Page no.
Fig. 1	Pharmacological Properties of *Selaginella Bryopteris*	3
Fig. 2	Steps involved in Research Methodology	12

TABLE OF CONTENT

S. No.	CONTENT	PAGE NO.
1.	INTRODUCTION	1-5
2.	REVIEW OF LITERATURE	6-9
3.	RESEARCH GAPS	10
4.	OBJECTIVES	11
5.	RESEARCH METHODOLOGY	12
6.	EXPECTED OUTCOMES OF THE RESEARCH	13
7.	REFERENCES	14-19

INTRODUCTION

Approximately 250,000 plant species are included in Kingdom Plantae, But the main challenging situation is that only 10% of these species have undergone clinical trials for the treatment of cancer (Usman et. al., 2022).

Since time immemorial plants have served human kind as major sources of medicinal agents and the indigenous knowledge of medicinal plants have been transmitted through centuries orally from generation to generation. Then the knowledge was formulated, documented and passed into the organized system of medicine, such as Ayurveda, Unani, Siddha and till today are used to cure specific ailments and these are the traditional systems in which 95% of the prescription were plant based (Satyavati et. al., 1987). They are often referred as phytochemicals, and these are the chemical compounds that generate a specific physiological effect on the human body (Edeoga et. al., 2005). The medicinal properties of plants are found in these chemical substances. All species of *Selaginella* herb are very useful resurrection plant species which are having recovery capabilities of therapeutic uses in traditional and modern system of medicine on large scale. Some species of *Selaginella* herb like as, *S. Tamariscina, S. Lepidophylla, S. Chrysocaulos, S. Bryopteris, S. Labordei*and *S. Moellendorffii* are noticed as promising medicinal uses in vitro antimicrobial, antiviral, antidiabetic, antimutagenic, anti-inflammatory, antinociceptive, antispasmodic and anticancer potentials because they contain potential components of various extracted chemical from plant included like flavonoids, phenyl propanoids, steroids, pigments, oxygen heterocycle, lignans, coumarins, quinoids, hormones, benzenoids, carbohydrates and alkaloids etc (Lee. et. al., 1995).

Selaginella Bryopteris (Linn.) or Sanjeevni Booti is widely known Indian medicinal plant which is called as "punjemariam". It is a lithophytic pteridophyte plant which is belonging to the family *Selaginellaceae*. It is mostly harvested in Uttarakhand, Uttar Pradesh, Orissa, Biharand the Aravalli Mountains of India in the months of July to September. The secondary metabolites that plants produces includes a wide variety of compounds, including alkaloids, phenols, flavonoids, tannins, sterols, glycosides, and many others (Sodipo et. al., 1991; Geetha et. al., 2014).

Taxonomical status or Scientific classification of the plant *Selaginella Bryopteris* given in Table 1 (Gautam et. al., 2023; Wadhwa et. al., 2023; Sivakumar et. al., 2023). It has been demonstrated

that the extracted active phytochemicals derived from a wide variety of plants have provided a therapeutic approach (Křížkovská et. al., 2021) respond well against cancer independently and in combination with other therapy approaches with authentic medicinal importance which continue to attract researchers to study the anti-cancer activities.

Phytochemistry also considers the structural composition of these metabolites as well as their industrial, commercial, and therapeutic applications (Pengelly et. al., 2020; Saxenaet. al., 2013). Terpenes, phenolic compounds, and alkaloids are the three main categories of SM found in plants (Ávalos García et. al., 2009). The flavonoids, alkaloids, saponins, terpenes, lignin, vitamins, minerals, taxanes, gums, biomolecules, glycosides, oils, and various other metabolites have been studied as an effective anti-cancer component because of their different mechanisms of action (Usman et. al., 2022).

Table 1. Taxonomical classification of *Selaginella Bryopteris*

Division	Lycopodiophyta
Super division	Embryophyta
Class	Lycopsida
Order	Selaginallales
Family	*Selaginellaceae*
Genus	*Selaginella*
Species	*Bryopteris L.*

The WHO, Traditional Medicine (TM) Strategy 2014-2023 aims to give potential to TM field's safety as well as the effectiveness along with the quality by enhancing the availability of understanding and offering guidelines upon regulation and standard quality assurance (Bhatt et. al., 2016).

Selaginella species have been shown to have a variety of beneficial pharmacological uses (Fig.1), including anti-carcinogenic properties, anti-stress properties, anti-diabetic properties, anti-depressant properties, antibacterial properties, antiprotozoal properties, antifungal

properties, and antiviral properties, among others (Paswan et. al., 2020).

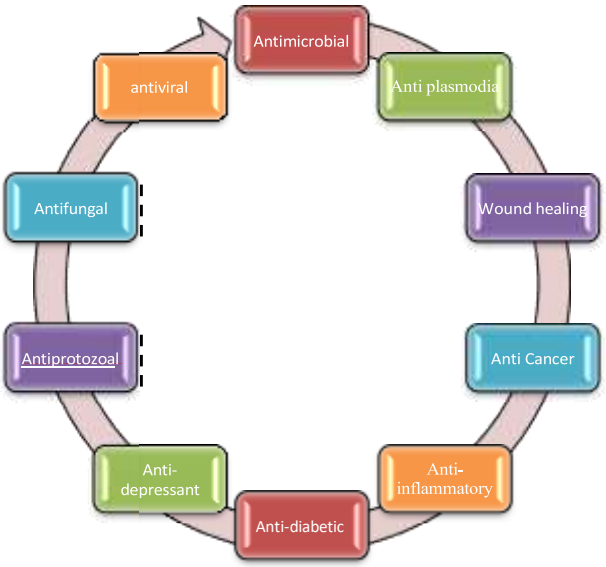

Fig 1. Pharmacological Properties of *Selaginella* Bryopteris

In previous studies, it has been proven from the clinical studies that phytochemicals such as curcumin, thymol, and rosmarinic acid are effective against bladder cancer, apoptosis, and colon carcinoma (Mustafa et. al., 2021). Even while "plant extracts" have been utilized in folk medicine for a long time and natural products are nontoxic to normal cells (Dijsselbloem et. al., 2004). WHO reported that, cancer is one of the major reasons of death affecting people worldwide and World health statistics 2019: monitoring health for the SDGs, sustainable development goals.

Chemotherapy and radiation therapy are the two current treatment choices available, while the surgical removal is occasionally chosen as well. The main drawbacks of radiation therapy are drug resistance, cancer recurrence, and damage to organs that are not being targeted. Therefore, it is vital to look for new lead anticancer medicines that have greater potency and fewer side effects. Even though there are many cancer treatment alternatives, research is continuing today to discover news substances from natural sources that are more effective in treating cancer or that may decrease the adverse impacts of therapy (Mera et. al., 2019).

Currently, the phytochemical research study is focused at isolation and characterization of

compounds synthesized or produced by plants having biological activities of prior for the therapy of human diseases because of their safety, cost-effectiveness and feasibility of oral administration (Raskin et. al., 2002). The biologically active molecules that an individual/people may utilize to his advantage are primarily found in plants. Such substances are mainly produced as a result of secondary metabolism's chemical reactions within the plants (Mera et. al., 2019; Weiß et. al., 2008). These chemicals react with particular targets in microorganisms or cells of animal to show biological effect that neutralizes them (Chen et. al., 2016; Cragg et. al., 2014). In addition to providing relief from heat stroke and the burning feeling that occurs during /urination, menstruation irregularities can be brought back to normal, pregnant women can have an easier birth (by reducing the amount of discomfort they experience during labor), and jaundice can be treated (Antony et. al., 2011).

For the purpose of determining pharmacological activity, potency, and toxicity, plant extracts can be assessed using a variety of biological approaches. The objective of qualitative and quantitative chemical analysis is to identify and separate the components that are responsible for the desired effects. The advancement of analytical instruments made so easy to quantitative determination of typical phytoconstituents. There is possibility to design more effective techniques for the standardization process by making a lot of advancements in the process of isolating, purifying, and elucidating the structure of naturally occurring metabolites. The homogeneity of a plant extract can be determined using techniques such as TLC, HPTLC, HPLC and GC. Methods such as spectroscopy with UV-Vis and FT-IR, mass spectrometry, gas chromatography with mass spectrometry (GC-MS), nuclear magnetic resonance (NMR), and electrophoresis are powerful tools that are frequently utilized for the purpose of standardization and quality control of both the raw material and finished product. The results recorded from these advanced procedures gave a chemical fingerprint that determines the types of compounds or contaminants are carried in the plant extract (Bilia et. al., 2002). The information that is gathered about these chemical ingredients not only helps in the process of finding novel medicinal medications, but it also has potential to assist in the disclosure of new sources of economic materials that are precursors for the synthesis of complicated chemical substances.

Their structures were clarified and comprehensive assignments of the 1H and 13C - NMR spectroscopic data were accomplished by the use of 1D and 2D NMR experiments on ROESY. With an IC(50) value of .5 3microg/ml, compound 1 antifungal activity against *Candia Albicans*

(Chikmawati et.al., 2012) determined the highest amentoflavone (6.87 ppm) content in based on aliphatic and oxygen containing moieties.

A study concluded that SPE@ Ag-NPs have the potential to be greatly utilized as a therapeutic agent in the field of biomedicine (Dakshayani et. al., 2019). An investigation into the effects of an excision wound healing model was proposed with the use of *S. Bryopteris* extract (Paswan et. al., 2020) in which they presented the outcomes which suggests the more clinical research should be conducted. This was followed by the evaluation of the total phenolic and flavonoid contents of the extract. GC-MS analysis was carried out in order to identify the active metabolites that were present in the extract.

Mythology behind the *Selaginella Bryopteris* (Linn.) plant

This herb, commonly referred to as "Sanjeevani," refers to "One that infuses life" and originates from the numerous therapeutic applications of the *Selaginella Bryopteris* plant. The Sanjeevani plant is described as a magical herb that has the capacity to remedy any illness, as stated in the epic Ramayana. It was also thought that medications made from this plant had the capacity to bring a person who had passed away back to physical existence. Sanjeevani explained the magical role that Ravana (Lankapati, DasisSakvithi, Dashaanan) played in the Ramayana. Indrajit (Meghnad), Ravana's son, used a formidable weaponry to attack Lakshmana (Laxman or Lakhan), the younger brother of God Rama. As a result, Lakshmana suffered severe injuries and was on the verge of being slain by Indrajita. It was then that Hanuman, a devoted follower of Lord Rama, went to Sushena, the Royal Physician of Lanka, to inquire about the circumstances surrounding Lakshmana and to seek his counsel. Hanuman, the son of Anjana, was given instructions by Sushena, the Lakhan Physician, to travel to the Dronagiri Hills. There, he was able to acquire four miraculous plants, which were named Mrutha sanjeevani, Vishalyakarani, Sandhanakarani, and Savarnyakarani. These plants were utilized for restoring life, removing arrows, and restoring skin and skin color, respectively. During the process of picking the four plants from the Dronagiri Hills, Hanuman became confused and ended up bringing the entire Dronagiri hill back with him. Lakshmana was able to restore his life after receiving therapy with the herb. According to the Ramayana and a number of scientific tests, it has been demonstrated that sanjeevani booti emits light and glows when it is stored in a dark environment (Antony et. al., 2011).

REVIEW OF THE LITERATURE

There is no doubt, this plant exhibits many different medical uses and has been revered as a mystical herb in the field of Pharmacognosy the locals due to its medicinal capabilities (Gautam et. al., 2023). Traditionally in the treatment of several health-related problems the *Selaginella Bryopteris* has been used but majorly remains to be scientific validation aspects (Pal et. al., 2022).

All species of *Selaginella* family has very wider range of activity for the treatment of spanning cancer, cardiovascular diseases, diabetes, gastritis, hepatitis, urinary tract infections and jaundice, dysentery, skin inflammation, fever and gynaecological problems (Ganeshaiah et. al., 2009; Antony et. al., 2011). *Selaginella* has been identified as a rich source of biflavonoids, lignans, alkaloids, terpenoids and anthraquinones etc (Setyawan et. al., 2008).

A research study has been done on relative dataset on the GC–MS and antioxidant effect of ***Selaginella Bryopteris*** from various geolocations of India (Srivastava et. al., 2025).

It was studied that by researchers at CSIR- National Botanical Research Institute in Lucknow, were searching for different and unique drought-resistant gene, and they were also interested to develop transgenic agricultural crops by putting its gene into the genomes with the help of biotechnology cereals like wheat, rice, and legumes. Additionally, if the monsoon fails, the cereal grains will not affected and could be able to survive the rough environment courtesy of Sanjeevani's gene (Gautam et. al., 2023).

This study elaborates the current and ancient pharmacological uses of S. Bryopteris. Additionally, some new ideas introduced to enhance the biological activity of S. Bryopteris through water solubility enhancements and medication combinations are presented by (Ren et. al. 2023).

Anticancer Activity of ***Selaginella Bryopteris*** studied by using silver nanoparticles, the use of various software and servers has yielded promising results indicating potential application of phytochemicals in Dendro biumplicatile as controllers against various ailments. Moreover the possible targets of phytochemicals have been unraveled through Swiss Target Prediction server and interaction with targets proved using docking simulations performed using iGem Dock server (Malik et al., 2021**;** Dutta et. al.,2025)**.**

The investigation, isolation and characterization of active components obtained from whole plant of *S. Bryopteris*. The petroleum ether, ethyl acetate and methanol were used as solvent system for extraction process. Several active phytochemical obtained like alkaloids, carbohydrates, reducing sugar, flavonoids, protein, amino acid, tannin and phenolic compound in methanolic extract of *S. Bryopteris* (Jain et. al., 2020).

Through the use of the Agar well diffusion method demonstrated that the methanolic extract of *S. Bryopteris* has the ability to inhibit the growth of harmful bacteria such as *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Bacillus subtilis, Escherichia coli, and Salmonella typhi* (Shukla et. al., 2017).

Quercetin isolation and characterization has been performed from *B. Alba's* whole plant and hexane, ethyl acetate, and ethanol extracts, Traditional qualitative methods were used for phytochemical analysis. Whole plant extracts were analyzed for TFCs (total flavonoid content). After being isolated, preparative HPLC, quercetin was analysed using mass spectrometry (MS), HPLC, ^{1}H NMR (proton NMR), ^{13}C NMR (carbon NMR), and others. The ethyl acetate extract had more flavonoids than the others. An entire study was reported on *B. Alba's* plant in which they extracted the first ethyl acetate from quercetin that showed the toxicological effects in female *Swiss albino* mice at dosages of 300 and 2000 mg/kg (Bose et. al., 2023).

The phytochemical screening and biological evaluations study of extracted components is must before any analysis, which delivers the basis for every research. For the chemical isolation of n-butanol extract the column chromatography were selected and after that identification of n-butanol was done by the UV-VIS spectrophotometer, FTIR, mass, and NMR spectroscopic analysis. The Study gave clarification on the series of extractions using different organic solvents for stems and roots of *Sesbania Sesban.* The n-butanol was most bioactive component that showed the presence of flavonoids, tannins, and coumarins. The chemicals that were recovered were identified as -B sitosterol and kaempferol. The information obtained from the chromatographic, ultraviolet, infrared, nuclear magnetic resonance, and mass spectrophotometer analysis, the substance that was separated from the extract, identified as kaempferol and ß-sitosterol (Kumaret. al., 2017).

Hexoses and proteins were the reported components from *Selaginella Bryopteris* that was identified from the chemical analysis (Deeba et. al., 2009). Moreover, the methanolic extract of

the plant was observed to hugely increase learning and memory in albino mice (Pandey et. al., 2010).

Study has been proposed to determine the physiological and biochemical changes that came in the fronds as a result of desiccation and rehydration. The main focus of this research is to get information of how this plant adjusts to the dry phase and continues to thrive. A research Study reported the powder of *Selaginella Bryopteris* was used in conjunction with tobacco for the purpose of hallucination (Dixit et. al., 1984).

The evolution of Phytopharmaceuticals derived from the phytochemicals of plants

The potentiality of bioactive components obtained from plants purely builds upon their ability to be used as medicinal advantage. The quality of phytochemicals directly influenced by some vital factors including the age, climate, and season of the plants, However, advance study is needed to boost our understanding of phytochemicals and how they might be utilize them in prevention and treatment of cancer.

In bioassay-guided fractionation, there are many advanced analytical methods available for purification and separation of phytochemicals from a complex of mixture of components. Preliminary, the biological activity of plant extract must be evaluated using either dry or wet plant material.

Several analytical methods are present that can be utilized for the fractionation and separation of bioactive material of plant extract like, mass spectroscopy, HPLC, TLC, FTIR, and NMR.

A huge range of solvents are available for use for separation method. For the fractionation, silica, superdex, and other appropriate matrices can be used. Variety of dyes can be used to identify the natural bioactive compounds in medicinal plants (Sebastian et. al., 2020). After purification process of phytochemicals, then these molecules are tested for structural confirmation by using different spectroscopic techniques and then went or pharmacological screening like in vivo or in vitro anti-cancer effects. Additionally, other aspects such as pharmacokinetics, metabolic fate, side effects, immunogenicity, pharmacodynamics, dose determination, and drug interaction are aimed on for prospect drug design.

Table 2. List of methods used for extraction, isolation and identification of plant *SelaginellaBryopteris.*

S.N	Part of plant used	Extraction and Isolation methods	No. of components isolated	No. of components observed	Technique used for isolation / characterization	References
1	Whole part, Dongargaon	Soxhlet extraction (Methanol)	Phytosterol, polyphenol, saponins, flavonoids and carbohydrates	-	-	Ansari et. al., 2023
2	Whole part, Aurangabad district	1. Aqueous extract 2. Ethanolic extract	Flavonoids, reducing sugar, anthocyanin, coumarin, steroid and alkaloid	Flavonoids and phenols (DPPH, ABTS and H2O2)	-	Prakash et. al., 2023
3	Spring - purulia district, west bengal	Ethyl acetate, acetone, chloroform, and methanol	-			Shibuet. al., 2022
4	Whole plant,VillageRainikheda and Tamiya, Dist. Chhindwara, (M.P.) India	Hot percolation method with Soxhlet apparatus (petroleum ether,ethyl acetate and methanol)	Phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins	Sitosterol by preparative, amentoflavone	TLC	Jain Abhilasha et. al., 2020
5	Fronds (leaves), stem, and roots from three different plants ,	-	-	-	-	Singh, Ravi S et. al., 2018
7	Leaves	Aqueous Extract				Abhishek Kumar et. al., 2017
8	Leaves and roots, (Western Ghats of South India)	Maceration and rotary evaporation-75ml of 70% ethanol (24 hours)	Vanillic acid, polyphenols,	Terpenoids,Polysaccharides like Chitosan, Gelatin and Pectin.	GCMS	Gautam, Arti et. al., 2023

RESEARCH GAPS

- ***Selaginella Bryopteris*** requires extensive research and clinical trials.
- Integrated Network Pharmacology + Experimental Validation — identify lead S. Bryopteris phytochemicals with anti-cancer targets and validate in vitro.
- Limited work done on purification method development & comprehensive chemical characterisation.
- Combine metabolite profiling (LC-MS & NMR) with network pharmacology (target prediction, pathway enrichment, docking) to prioritize candidate phytochemicals from *S. Bryopteris*
- Rather than using crude plant extract, we can isolate individual HPLC peaks (single compounds or well-defined fractions), identify them (NMR, HR-MS)
- Limited analytical techniques used before because of the availability of advanced analytical techniques and equipment for isolation, purification & identification.
- Requires the safety and toxicity studies.
- Efficiency/ Efficacy studies.

OBJECTIVES

1. Extraction of phytochemicals from whole plant ***Selaginella Bryopteris***.
2. Comprehensive isolation and structural elucidation of bioactive compounds using **preparative chromatography, NMR**, **LCMS, GCMS,** and **HR-MS** etc.
3. Validate top candidates for Structural and biological characterization of new phytochemicals and potential therapeutic applications.

RESEARCH METHODOLOGY

1. Sequential extraction method will employed to extract the plant powders using different organic solvents from *low polar to high polar* namely hexane, chloroform, ethyl acetate, and methanol.
2. Standardize collection and analytical fingerprinting of *S. Bryopteris*, establishing reproducible extraction.
3. Preparative HPLC protocols will be employed and generating an LC-MS/HRMS reference library.
4. Representative analytical HPLC fingerprint + LC-MS base peak chromatogram.
5. Focus on the isolation and structural elucidation by using 1D/2D NMR and HRMS techniques of some major phytochemicals peaks (flavonoids, biflavonoids, glycosides and small phenolics).
6. Prepare a library of number of peaks targeted, yields, and purity criteria.
7. Validate top candidates for biological evaluation and establishing its therapeutic potential.

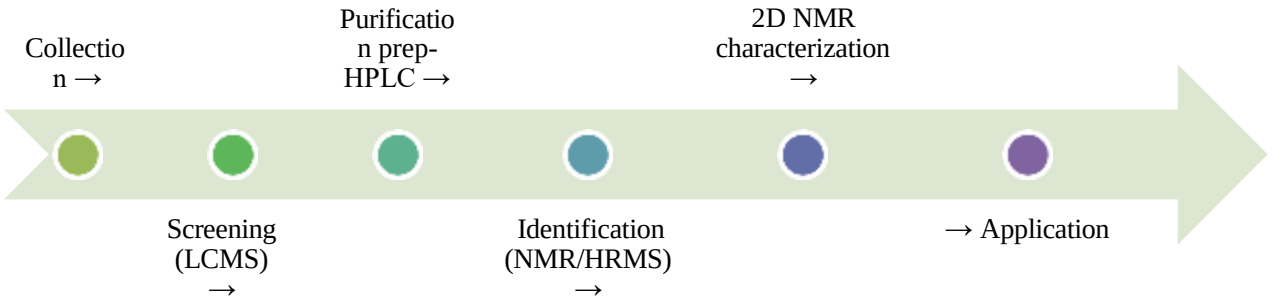

Fig.2 Steps involved in Research Methodology

EXPECTED OUTCOMES

- Full phytochemicals profile of *Selaginella Bryopteris* using LC-MS and NMR data.
- Isolation & structure elucidation of key bioactive compounds (flavonoids, biflavonoids, alkaloids, lignans).
- Correlation between phytochemicals & bioactivity (antioxidant, anti-inflammatory, anti-aging assays).
- Research database for further work (e.g. nanoparticle synthesis, formulation development).

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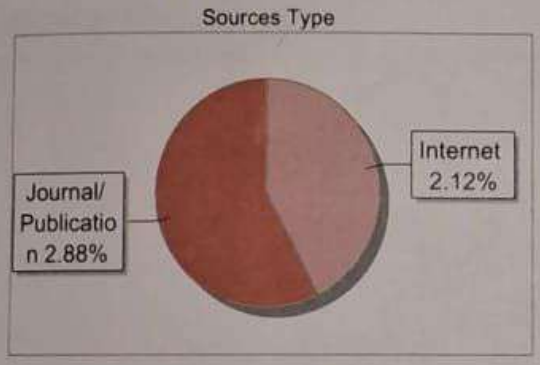

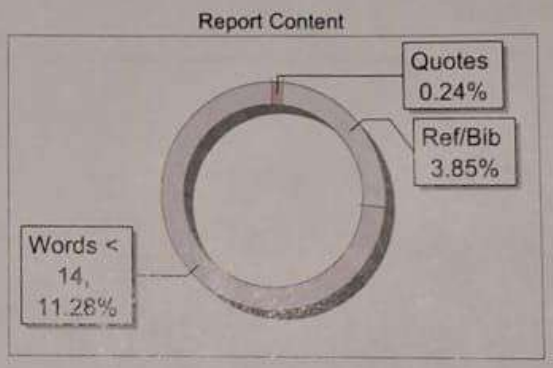

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Screening, Prep-HPLC Isolation and Chemical Characterization of Potential Bioactive Compounds Derived from *Selaginella Bryopteris* Using Advanced Analytical Tool

SHORT SYNOPSIS

Submitted in partial fulfillment of the requirement of the degree of

DOCTOR OF PHILOSOPHY

by

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ABSTRACT

Phytochemicals are bioactive substances that exist naturally in plants. The *Selaginella Bryopteris* is a member of the family *Selaginellaceae*, which is also referred to as "Sanjeevani." This research aims to explore the development of new analytical methods to improve isolation, purification and structure elucidation of potential bioactive compounds of *Selaginella Bryopteris* through comprehensive study. The study shall include preparative HPLC for the effective isolation of the plant's constituents, followed by detailed structural elucidation using NMR and Hyphenated-MS techniques. Furthermore, AI tools shall be used to predict the biological activities of the isolated compounds, focusing on potential antimicrobial, anti-inflammatory, antioxidant and oncological effects. This innovative approach seeks to bridge traditional knowledge and improves the understanding with modern technology, potentially uncovering novel Phytopharmaceuticals and enhancing our understanding of *Selaginella Bryopteris* therapeutic applications in modern era.

Keywords: Phytochemicals, *Selaginella Bryopteris*, isolation, Prep HPLC, NMR, MS.

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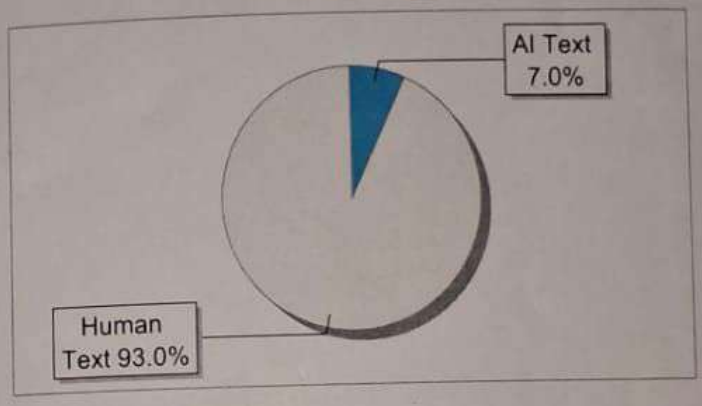

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